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Phaeostilbelloides and *Velloziomyces* — new dematiaceous genera from the Brazilian Cerrado

Eliane Amaral de Souza Armando,
Zuleide Martins Chaves, & José Carmine Dianese*

Departamento de Fitopatologia, Universidade de Brasília,
Campus Darcy Ribeiro, Asa Norte, 70910-900 Brasília, DF, Brazil

* Correspondence to: *jcarmine@gmail.com*

Abstract —*Phaeostilbelloides velloziae* gen. & sp. nov. and *Velloziomyces ramosiconidialis* gen. & sp. nov. are described and illustrated. Both new fungi infect *Vellozia squamata*, endemic to the Brazilian Cerrado.

Key words — neotropical fungi, asexual ascomycete morphs, mycodiversity, *Velloziaceae*

Introduction

Starting in 1993, an extensive inventory of fungi associated with plants native to the Brazilian savanna, designated as the Cerrado, (Dianese 2000, Dianese et al. 1993b, 1997) yielded important records of unusual hyphomycetes such as those inhabiting the trichomes of Cerrado plants (Cantrell et al. 2011, Pereira-Carvalho et al. 2009b). To date, 13 new ascomycete genera have been described, ten as asexual morphs (Dornelo-Silva & Dianese 2004, Pereira-Carvalho et al. 2009b) and three as sexual morphs (Dianese & al. 2001, Pereira-Carvalho 2009a, Pereira-Carvalho et al. 2010). In addition, two new genera of rust fungi, *Batistopsora* and *Kimuromyces* (Cummins & Hiratsuka 2003, Dianese et al. 1993a, 1995), have been described and a third, *Mimema*, has been reinstated (Cummins & Hiratsuka 2003, Dianese et al. 1994). Several cercosporoid fungi (Dianese et al. 2008, Dornelo-Silva et al. 2007, Furlanetto & Dianese 1999, Hernandez-Gutierrez & Dianese 2008, 2009, 2014, Inacio & Dianese 2006, Medeiros & Dianese 1994) have been described and other new ascomycetes have been included in other publications (Dornelo-Silva & Dianese 2003, Inacio et al. 2011, 2012, Soares & Dianese 2014, Souza et al. 2008). This paper expands the number of genera among the asexual ascomycete

morphs from the Cerrado, with the description and illustration of two new genera, *Phaeostilbelloides* and *Velloziomyces*.

Materials & methods

Fungi associated with plants native in the Cerrado Biome were extensively collected. Observations under a stereomicroscope revealed the presence of dematiaceous hyphomycetes on leaves of *Vellozia squamata* Pohl (*Velloziaceae*), which is a Brazilian Cerrado endemic. Squash preparations were mounted on semi-permanent slides stained with lacto-glycerol/cotton blue or glycerol-KOH/basic phloxine. Mounting media without stains were also used to determine the color of different structures. Sections about 15–25 µm thick were produced using a Leica CM 1520 freezing microtome. The structures observed in the squash preparations and sections were measured, described, and documented using a Leica DM 2500 microscope coupled with a Leica DFC 490 digital camera connected to a microcomputer. Image capture, editing, and measurements were made with Leica QWin V3 software. Whenever possible 20–50 measurements were made and expressed as range of length by width, with the mean measures indicated in parentheses.

Taxonomy

Phaeostilbelloides Armando, Z.M. Chaves & Dianese, **gen. nov.**

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Differs from *Phaeostilbella* by its lack of rigid setae on the conidiomata.

Type species: *Phaeostilbelloides velloziae* Armando et al.

Etymology: Latin *Phaeostilbelloides,* meaning similar to the genus *Phaeostilbella.*

Conidiomata synnematous, scattered, erect, determinate, dark brown to brown. Conidiophores macronematous, cylindrical, branched just below the apex, septate, straight or flexuous, brown. Conidiogenous cells monophialidic, integrated, terminal, determinate. Conidia acrogenous, cylindrical to ellipsoid, unicellular, brown or pale brown.

Phaeostilbelloides velloziae Armando, Z.M. Chaves & Dianese, **sp. nov.** Plate 1

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Differs from *Phaeostilbella* species by its lack of rigid curved setae on the conidiomatal top and differs from *Myrothecium* species by its dark brown or brown conidiophores and conidiogenous cells and its lack of a sporodochial phase.

Type: Brazil. Distrito Federal: Brasília, West Sector of the IBGE-Roncador Reserve, on living leaves of *Vellozia squamata*, 5 Apr. 2001, J.C. de Castro 82 (**Holotype,** UBMC 18404).

Etymology: Latin *velloziae*, referring to the host genus.

Lesions necrotic, linear, elongate, 5–10 × 0.5 mm, following parallel leaf veins. Mycelium immersed; hyphae light brown, septate 3–5 µm wide.

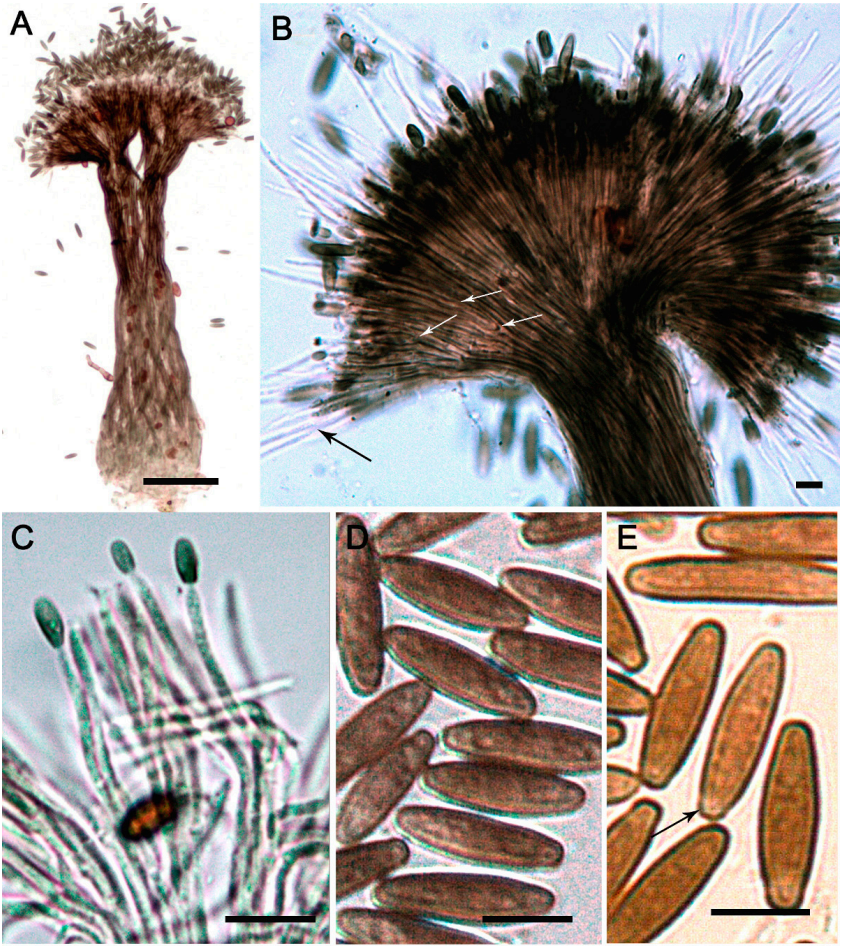


Figure 1 *Phaeostilbelloides velloziae* (holotype, UBMC 18404) on leaf of *Vellozia squamata*. A. Synnema showing stipe with textura intricata; B. Synnema top showing subapical branching (white arrows), and abundant hyaline proliferations of the conidiophores (black arrow); C. Conidiogenous cells and conidia; D. Conidia; E. Conidia with clearer area (arrow) at the conidial base. Scale bars: A = 50 µm; C = 10 µm; B, D, E = 5 µm.

Conidiomata 194–(212)–294 × 54–(56)–60 µm, synnematous cylindrical, stipe textura intricata, smooth, dark brown, expanded to a 150–(166)–175 µm wide brush-like capitulum, containing large number of immature conidiophores that show up in squash preparations. Conidiophores 3–5 µm

in diam., cylindrical, smooth, originating as sub-terminal branches at the capitulum, reduced to a single cell bearing two conidiogenous cells. Conidiogenous cells monophialidic, integrated, terminal, cylindrical, 10–(15)–20 µm long, 1–3 µm in diam., showing clear periclinal thickenings but not a collarette. Conidia 8–(7.5)–11 × 2–(2.5)–3 µm, noncatenate, aseptate, sub-cylindrical, with an obtuse apex and truncate base, brown to dark brown, with a slightly clear band just above the hilum, accumulating in mucilaginous, dark brown masses.

Additional specimen examined: **BRAZIL. Distrito Federal**: planaltina, Reserva Ecológica Águas Emendadas, on living leaves of *Vellozia squamata*, 25 Mar. 1995, Mariza Sanchez 530 (UBMC 7668).

Comments – Following the concepts by Seifert (1985) and Seifert et al. (2011), it is not possible to place the specimen in any of the known genera producing synnematous conidiomata, 1-celled brown to dark-brown conidia, and phialidic conidial ontogeny. The closest genus, *Myrothecium* Tode [= *Solheimia* E.F. Morris] as described and illustrated by Seifert et al. (2011), shares some characteristics in common with the new taxon. However, the differences between *Phaeostilbelloides velloziae* and *Myrothecium* species are sufficient to place them in two separate genera. All *Myrothecium* species produce dark smooth conidia, except for one species that shows conidia with strongly striate surface and fusiform with acute ends. In addition, the synnematous conidiomata are unbranched, and sporodochial conidiomata may also be present. In contrast, *P. velloziae* lacks sporodochia, produces brown to dark brown smooth-walled cylindrical conidia with an obtuse apex and a pale area just above the truncate base, and synnemata that are sometimes branched. Another synnematous species described from Brazil, *Chaetantromycopsis bambusae* H.P. Upadhyay et al., produces black slimy spore heads but clearly differs from *Phaeostilbelloides* by its hyaline to creamy white conidiophores and the presence of erect acute hyaline to creamy white setae encircling the base of the synnemata (Upadhyay et al. 1986). Finally, the *Phaeostilbelloides* type species is easily separated from *Phaeostilbella* species because the hyaline cylindrical conidiophore branches seen at the top in squash preparations differ completely from the rigid slightly curved appendices of *Phaeostilbella*. Furthermore, *Phaeostilbella* synnemata typically show textura prismatica, not textura intricata as in *Phaeostilbelloides*. Also, the only species currently accepted in *Phaeostilbella* was reported on grasses in Europe (Seifert et al. 2011) while *Phaeostilbelloides velloziae* is present in a dicotyledonous host endemic to the Cerrado.

The dichotomous key below, modified from Seifert et al. (2011), segregates *Phaeostilbelloides* from all other genera with synnematous conidiomata forming phialidic conidiogenous cells bearing 1-celled conidia.

Key to the dematiaceous genera of phialidic synnematous anamorphs forming 1-celled conidia

1. Conidia hyaline, globose to ellipsoidal, ovoid, fusoid, in clear or bright colored mucilaginous false heads. 2
1. Conidia brown to dark brown, cylindrical, fusoid, in dark brown to black false heads 7
2. Synnemata originating from broad well developed stromata, found on wood . . . . . *Stromatographium* Höhn.
2. Synnemata lacking a well-developed basal stroma. 3
3. Synnemata with ornamenting cells on stipe . . . . . anamorphic *Stilbocrea* Pat. (= *Gracilistilbella* Seifert)
3. Synnemata without ornamenting cells on the stipe 4
4. Synnematous heads with sterile hyphae among the conidiogenous cells . . . . . anamorphic *Holwaya* Sacc. (= *Crinula* Fr.)
4. Synnematous heads lacking sterile hyphae 5
5. Conidiophores penicillate occurring in pustules on the upper portion of the stipe . . . . . *Cornutostilbe* Seifert
5. Stipe without conidiophores in pustules 6
6. Conidiogenous cells lageniform with characteristically narrow elongated necks, conidia obovoid. . . . . .*Dennisographium* Rifai
6. Conidiogenous cells holoblastic or phialidic terminal at the tip of each conidiophore, lacking narrow neck. . . . . .*Synnemellisia* N.K. Rao et al.
7. Synnemata with setose capitulum, showing a crown of dark setae, conidia fusoid . . . . . .*Phaeostilbella* Höhn.
7. Synnemata lacking setae at capitulum or with subhyaline hyphae 8
8. Capitulum without sterile hyphae, conidia fusiform, with smooth or striate surface, sporodochial phase often present. . . . . .*Myrothecium* (= *Solheimia*)
8. Capitulum non-setose but with subhyaline hyphae, conidia cylindrical, with obtuse apex and pale band just above the truncate base, sporodochial phase absent . . . . . .*Phaeostilbelloides*

Velloziomyces Armando, Z.M. Chaves & Dianese, **gen. nov.**

MycoBank MB808835

Differs from *Minimidochium* by its holoblastic conidial ontogeny and from *Dictyodochium* by its schizolytic conidial secession.

Type species: *Velloziomyces ramosiconidialis* Armando et al.

Etymology: From the Latin *Velloziomyces* for its occurrence on *Vellozia*.

Conidiomata sporodochial scattered or confluent, setose, dark brown, Setae cylindrical, erect, straight or flexuous, continuous or septate, brown to dark brown. Conidiophores undifferentiated Conidiogenous cells monoblastic, cylindrical to lageniform, indeterminate, with several enteroblastic percurrent

extensions, integrated, terminal. Conidia solitary, obclavate or Y-shaped, 0–2-septate, simple or branched, brown to pale brown.

Velloziomyces ramosiconidialis Armando, Z.M. Chaves & Dianese, **sp. nov.**

MycoBank MB808836 Plates 2, 3

Differs from *Minimidochium* species by its holoblastic conidial ontogeny, from *Dictyodochium* species by its schizolytic conidial secession of phragmospores, and from *Iyengarina* species by its setose sporodochial conidiophores and association with living leaves.

Type: Brazil. Distrito Federal: Planaltina, Estação Ecológica Águas Emendadas, on living leaves of *Vellozia squamata*, 13 Jun. 1995, Arenildo Soares Alves 215 (**Holotype,** UBMC 8849).

Etymology: From the Latin *ramosiconidialis*, referring to conidial branching.

Colonies amphigenous, light brown, erumpent, setose. Mycelium mostly immersed, composed of septate, light brown, smooth hyphae. Conidiomata sporodochial suprastomatal, scattered or confluent, setose, dark brown, 10–(42)–55 µm diam. Setae cylindrical, erect, straight or flexuous, sometimes slightly curved, obtuse or acerose at the apex, continue or septate, brown to dark brown, 60–(112)–138 × 3–(4)–6 µm. Conidiogenous cells monoblastic, indeterminate, with several enteroblastic percurrent extensions, terminal, short cylindrical, lageniform 3–9 × 3–5 µm. Conidia solitary, 1–2-septate, more or less Y-shaped, with a lateral branch, obclavate at main axis (7–(18)–25 × 3–(8)–16 µm) and one shorter lateral branch, light brown, with paler conical apical cell, truncate base.

Additional specimen examined: **BRAZIL. Distrito Federal**: Brasília, Jardim Botânico de Brasília, on living leaves of *Vellozia squamata*, 24 May 2011, Eliane Armando 129 (UBMC 21928).

Comments – Sporodochial conidiomata are distributed in several genera of dematiaceous anamorphs (Ellis 1971, Ellis 1976, Kiffer and Morelet 2000, Seifert 1985, Seifert et al. 2011). However, there is no published genus described as producing setose sporodochia on top of an obconical stromatic structure that grows through the stomata. As illustrated here, the stroma originates deep within the leaf parenchyma, grows through the stoma to expand at the surface into a multicellular plate formed by a layer of tightly packed annellidic conidiogenous cells surrounded by dark brown elongated setae. In addition, the light brown conidia are unusual because at maturity they have one unicellular conical branch growing from the basal cell, which is shorter than the main 2–3-celled axis of the spore. Sometimes the branch is not present.

Two genera, *Minimidochium* B. Sutton and *Dictyodochium* Sivan., as shown in Seifert et al. (2011), have setose sporodochial conidiomata that originate from internal mycelium emerging from plant tissue, as in *Velloziomyces*.

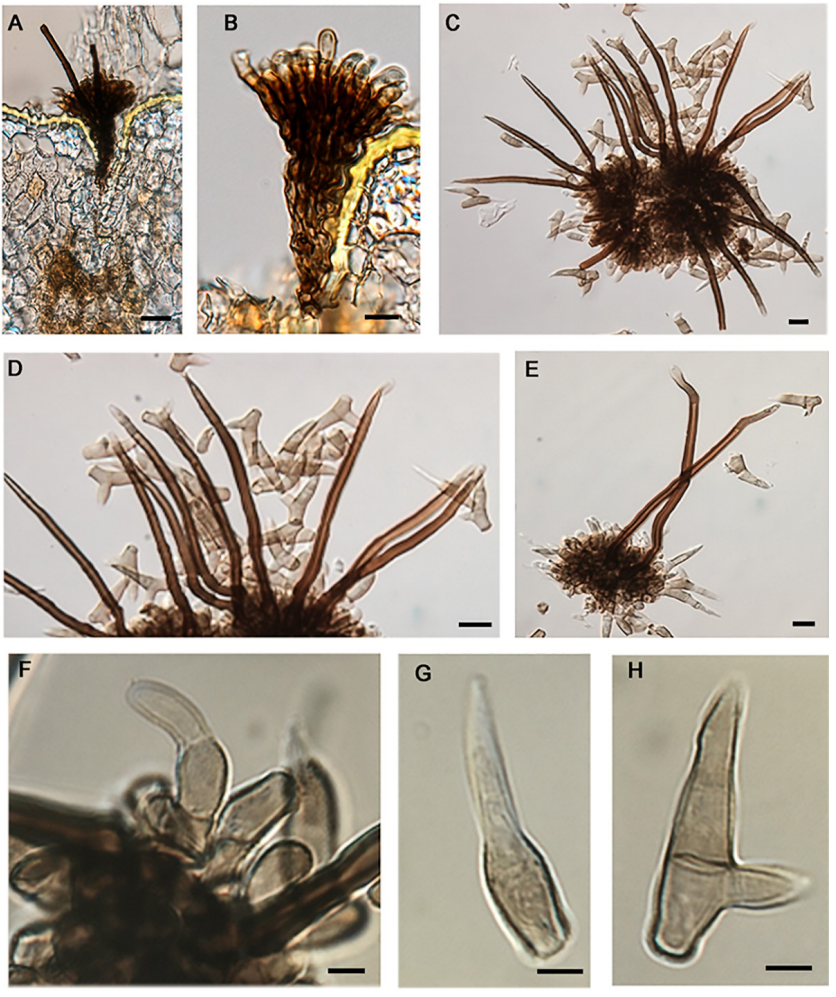
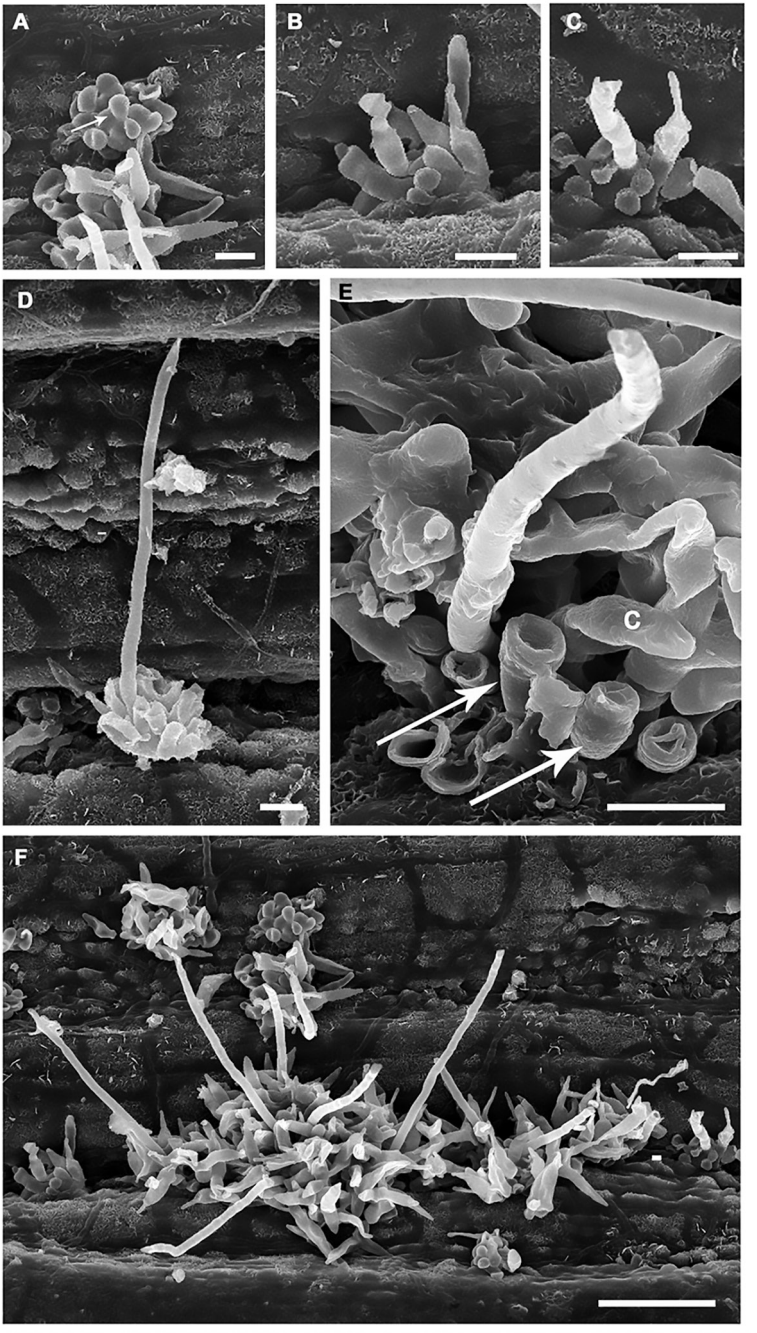


Figure 2 *Velloziomyces ramosiconidialis* (holotype, UBMC 8849) on leaf of *Vellozia squamata*. A. Setose stromatic sporodochium; B. Sporodochium detail of the superficial distribution of the conidiogenous cells; C. Setose sporodochium bearing conidia; D. Setae detail and a group of free conidia; E. Setose sporodochium; F. Conidiogenous cell; G–H. Conidia focused in different depths. Scale bars: A = 20 µm; B–E = 10 µm; F–H = 5 µm.

However, all eight species of *Minimidochium*: *M. crepuscolare* C. Ciccar., *M. eucalypti* Vittal & Dorai, *M. indicum* Varghese & V.G. Rao, *M. microsporum* Matsush., *M. monoseptatum* G.C. Zhao, *M. nipponicum* Matsush., *M. parvum*


A
B
C
D
E
C
F

Cabello et al., and *M. setosum* B. Sutton (Cabello et al. 1998, Ciccarone 1988, Matsushima 1995, Sutton 1969, Varghese & Rao 1980, Vittal & Dorai 1991, Zhao & Zhao 2012) produce phialidic conidiogenous cells bearing typically setulose hyaline conidia. On the other hand, in the monotypic genus *Dictyodochium*, the muriform brown conidia have rhexolytic secession. Also, the three species belonging in *Iyengarina* Subram. (Kuthubutheen & Nawawi 1992, Subramanian 1958) are easily segregated from *V. ramosiconidialis* due to clear differences in conidial morphology, inability to form setose sporodochial conidiomata, and association with plant debris instead of living leaves. Thus, species belonging in all three genera clearly differ from *V. ramosiconidialis,* which is characterized by its holoblastic annellidic conidiogenous cells, non-setulose branched conidia showing schizolytic secession, and setose sporodochial conidiomata.

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Figure 3 *Velloziomyces ramosiconidialis* (holotype, UBMC 8849) on leaf of *Vellozia squamata* (SEM). A–C. Developing sporodochium seen from the top; D. Sporodochium showing a mature seta; E. Setose sporodochium and conidiogenous cells (arrows), and a conidium (C) still attached to the conidiogenous cell; F. Top view of a typical setose sporodochium. Scale bars: A–E = 10 μm; F = 40 μm.

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